**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Patent Application No. 09/463,890

Confirmation No. 6925

Applicant: Koszinowski et al.

Filed: April 28, 2000

TC/AU: 1636

Examiner: Fereydoun Ghotb Sajjadi

Docket No.: 203676 (Client Reference No. PA29186USFZSEhud)

Customer No.: 23460

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 C.F.R. § 1.132 OF MARTIN MESSERLE**

I, Martin Messerle, do hereby declare:

1. I am a co-inventor of the above-referenced patent application. I am Associate Professor for Molecular Virology at the Hannover Medical School. A copy of my *curriculum vitae* is enclosed herewith.

2. I have read Chartier et al., *J. Virol.*, *70*: 4805-4810 (1996), which is cited in the Office Action dated December 30, 2008.

3. The method disclosed in the Chartier reference is not suitable for generating virus genomes that are larger than an adenovirus genome (i.e., 36 kilobases) for the reasons described below.

4. The plasmid vector used in the Chartier reference would not support the stable replication of DNA fragments that are larger than 100 kb. Indeed, vectors comparable to those disclosed in the Chartier reference (e.g., pBR322, pACYC177, and pACYC184 [Chang and Cohen, *J. Bact.*, *134*: 1141-1156 (1978)]) have an upper limit cloning capacity of around

40 kb (see, e.g., Casali, N. and Preston, A.; *Methods in Molecular Biology. Vol. 235:* E.coli Plasmid Vectors; Humana Press Inc., Towata, NJ (2003); pp. 19.
and also Sambrook, J. and Russell, D.W. Molecular Cloning. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2001); Chapter 1, 1.1-1.20 and Chapter 4, 4.1-4.10).

3. The genome structure of herpes viruses is complex, and contains numerous direct and indirect sequence-repeats. The pTG3602 construct disclosed in the Chartier reference was stable due to the insertion of the 2 kb ppolyII sequence between the two viral ITRs, which prevented the formation of palindromic sequences (Chartier reference at page 4806, right column, last sentence of 1st ¶). In view of the multitude of direct and indirect repeats present in the herpes virus genome, a herpes virus genome contained in a BAC would not be supported by the *E.coli* recBC sbcBC strain used in the Chartier reference.

4. Most herpes viruses (e.g., cytomegalovirus, herpes simplex virus, etc.) contain an identical terminal repeat (the "a-sequence") at the respective ends of the genome. The a-sequence is essential for the cleavage and packaging of the viral genome. Inclusion of the a-sequence in a region targeted for a recombination event, as performed in the Chartier reference, likely would result in cloning of only the a-sequence, rather than the entire virus genome. In addition, the *a* sequence occurs multiple times in the rest of the genome (within the internal repeat). It follows from this multiple occurrence of the *a*-sequence that the cloned genome would be unstable in the strain used by Chartier, or that only a subfragment of the genome could be obtained. Briefly, Chartiers' technique is sensible towards repetitive sequences. Herpes virus genomes contain multiple repetitive sequences which are essential to herpes viral function and can not be omitted.

5. The a-sequences are furthermore flanked by additional repetitive sequences known as the b- and c-repeats. Therefore, extending the region of the herpes virus genome that may be used for a homologous recombination event, which requires unique sequences to be accurate, would not compensate for any of the deficiencies described above. The Chartier reference discloses that approximately 1 kb of unique sequence must be present at the respective ends of the genome to be cloned (see Chartier reference at Figure 1A, top panel).

Such regions of unique terminal sequences are not available in herpes viruses. See, e.g., Roizman, "The Family Herpesviridae: A brief introduction," and Roizman and Spears, "Herpes Simplex Viruses and Their Replication," both in: Fields et al. (eds.), *Fields Virology, 3rd Ed.*, Lippincott, Williams & Wilkins (1996).

6. The Chartier reference discloses using *E. coli* strain BJ5183 for the recombination of the plasmids containing the Ad5 genome. BJ5183 has the genotype recB recC sbcB sbcC endA galK met thi-1 bioT hsdR rpsL(strR). In contrast, the *E. coli* strain disclosed in the present application for the maintenance of the claimed BACs is *E. coli* HB10. The HB10 strain has the genotype F- endA1 recA1 galE15 galK16 nupG rpsL ΔlacX74 Φ80lacZΔM15 araD139 A(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC) λ-. Chartier's technique requires the use of this strain (Figure 1 A, "homologous recombination in recBC sbcBC *E.coli*"). Hence, a strain as used in the present invention (which is not recBC sbcBC) is not suitable for carrying out the technique of Chartier.

7. In addition, the technique disclosed in the Chartier reference is not suitable for carrying out the present invention, because the technique disclosed in the Chartier reference requires the transformation of a particular *E.coli* strain with a linear virus genome. The particular *E. coli* strain disclosed in the Chartier reference is not defective in *E. coli*'s endogenous restriction endonuclease system. Therefore, transformation of the Chartier strain with a linear herpes virus genome three times larger than the Ad5 genome would lead to fragmentation of the genome through the action of the restriction endonuclease system.

8. The handling of linear DNA fragments (> 100 kb) is difficult and prone to single- and double-strand breakages of the DNA. It is uncertain whether an infectious linear herpes virus DNA could be produced in sufficient quality to effect a transformation of *E. coli* with the entire linear fragement. Furthermore, it is highly uncertain that a linear DNA greater than 100 kb can be efficiently transformed into *E. coli*. The Chartier reference acknowledges these limitations when it states that "*the size of the Ad5 genome (36 kb) constitutes a limiting factor for the efficiency of transformation*". This possibility is supported by the observation that *E. coli* transformation is inhibited when increased amounts of Ad5 DNA are used (Table 1)" (Chartier reference at paragraph bridging pages 4805-4806, emphasis added).

9. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 04/27/2009

Martin Messerle

Attachments: Curriculum Vitae

# CV PROF. DR. MARTIN MESSERLE


| | |
|---|---|
| Name: | Prof. Dr. rer. nat. Martin Messerle |
| Born: | March 08, 1959 in Hochdorf (Baden-Württemberg), Germany |
| Citizenship: | German |
| Home address: | Thieshof 3, 30659 Hannover, Germany |
| Work address: | Department of Virology, Hanover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany<br>phone: ++49 511 532 4320<br>e-mail: messerle.martin@mh-hannover.de |


## Education / Degrees

| | |
|---|---|
| 1980 – 1986 | Studies of Biology; University of Tübingen, Tübingen, Germany |
| 1986 | Diploma in Biology (equivalent to Master of Science) |
| 1986 – 1990 | Ph.D. thesis at the Research Center for Virus Disease of Animals, Tübingen and Department of Virology, University of Ulm |
| 1990 | PhD (Dr. rer. nat) from the University of Tübingen |
| 2002 | Habilitation in Experimental Virology, Ludwig-Maximilians-University of Munich, Germany |

## Positions held

| | |
|---|---|
| 1990 - 1992 | Postdoctoral fellow, Department of Virology, University of Ulm |
| 1993 – 1994 | Postdoctoral fellow at the Department of Haematology and Oncology, Division of Molecular Medicine, University of Freiburg |
| 1994 – 1996 | Scientific Assistant at the Department of Virology, University of Heidelberg |
| 1997 | Guest scientist at the Scripps Research Institute, La Jolla, CA, USA |
| 1996 – 2001 | Scientific Assistant at the Max von Pettenkofer-Institute, Gene-Center, University of Munich |
| 2001 – 2004 | Head of the Virus-Cell-Interaction Group, Medical Faculty, University of Halle-Wittenberg |
| since 2005 | Associate Professor for Molecular Virology, Hannover Medical School |

Martin Messerle

Hannover, April 27, 2009

Prof. Dr. Martin Messerle